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# 慈溪麦冬甙 A 和 B 的结构*

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**摘要：从浙江慈溪产中药麦冬（*Ophiopogon japonicum*）中分离得到 2 个新的 $C_{27}$甾体甙——慈溪麦冬甙 A（2）和 B（3）以及已知甙 ophiogenin 3－氧－α－L－鼠李糖(1→2)－β－D－葡萄糖吡喃甙（1）。根据光谱和化学证据推定 2 个新化合物的结构为 ophiogenin 3－氧－α－L－鼠李糖吡喃基（1→2)[β－D－木糖吡喃基(1→3)]－β－D－葡萄糖吡喃甙（2）和 ophiogenin 3－氧－α－L－鼠李糖吡喃基（1→2）[β－D－木糖吡喃基（1→3)] [β－D－葡萄糖吡喃基（1→4)]－β－D－葡萄糖吡喃甙（3）。**

**关键词：百合科；麦冬；$C_{27}$甾体甙；慈溪麦冬甙 A，B**
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# Cixi－ophiopogon A and B, $C_{27}$ Steroidal Glycosides from *Ophiopogon japonicum*

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**Abstract:** Two new $C_{27}$ steroidal glycosides nominated cixi－ophiopogon A (**2**) and B (**3**) together with a known ophiogenin 3－O－α－L－rhamnopyranosyl (1→2)－β－D－glucopyranoside (**1**) were isolated from the tubers of famous traditional Chinese herb － *Ophiopogon japonicum*. The spectroscopic and chemical evidences revealed their structures to be ophiogenin 3－O－α－L－rhamnopyranosyl (1→2) [β－D－xylopyranosyl (1→3]－β－D－glucopyranoside (**2**) and ophiogenin 3－O－α－L－rhamnopyranosyl (1→2) [β－D－xylopyranosyl (1→3] [β－D－glucopyranosyl (1→4)]－β－D－glucopyranoside (**3**), respectively.

**Key words:** Liliaceae, *Ophiopogon japonicum*, $C_{27}$ steroidal glycosides, Cixi－ophiopogon A and B

The tuber of *Ophiopogon japonicum* is recorded to impose various functions, such as against cardiovuscular diseases and anti－bacteria, and used as a potent drug to treat different diseases, especially heart diseases in the Oriental Medicine System (**江苏新医学院编**, 1977). Therefore, much more attention has been paid to the studies of chemical components of *O. japonicum* in recent decades since the first steroidal glycoside was isolated from the plant by

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Japanese scholars (Tada *et al*, 1972). Up to date, besides borneol and its glycosides (Adinolfi *et al*, 1990), homoisoflavonoids (Tada *et al*, 1980; 朱永新, 1987) and steroidal glycosides as the major glycosides with the aglycones of ruscogenin and diosgenin had been reported (Tada *et al*, 1973; Nakanishi *et al*, 1987; Adinolfi *et al*, 1990; Branke *et al*, 1995). Because of our interest in the development of preparation SHENG－MAI－SAN which was composed of *Panax ginseng*, *O. japonicum* and *Schisandra chinensis*, *O. japonicum* native to different places in China has been investigated in order to examine the bioactively chemical components and to prepare authentic steroidal glycosides for assay control of drug. Two new steroidal glycoside named cixi－ophiopogon A (**2**) and B (**3**) possessing ophipogenin as aglycone, alone with a known compound－ophiogenin 3－O－α－L－rhamnopyranosyl (1→2)－β－D－glucopyranoside (**1**) (Adinolfi *et al*, 1990) as its major glycoside were isolated from *O. japonicum* collected in Cixi County, Zhejiang Province, P. R. China in September, 1995. Herein, the structural elucidation on the basis of the combined spectroscopic and chemical means is described.

Cixi－ophiopogon A (**2**) corresponded with a molecular formula $C_{44}H_{70}O_{18}$ which was determined from the quasi－molecular ion peak at m/z 885.4549 $[(C_{44}H_{70}O_{18})-H]^-$ in its negative ion high－resolution FAB－MS (HR－FAB－MS) measurement. The IR of **2** showed a characteristic of 25 (R)－spirosteroid at 980, 930, 910 and 870 $cm^{-1}$ (the intensity: 910＞930). The $^1H$ NMR spectrum of **2** demonstrated (See Experimental) a pattern very similar to that of compound **1** (Adinolfi *et al*, 1990) except for one more hexose, and the coupling constants of the anomeric proton signals at δ 4.38 (1H, d, J = 8.1 Hz), 4.93 (1H, d, J = 7.8 Hz), and 6.16 (1H, br. s) indicated two β－linkages and one α linkage in the sugar chain. On acidic hydrolysis, **2** gave ophiogenin (**4**), glucose, rhamnose and xylose by TLC and PC comparison with authentic samples. Accordingly, the negative ion FAB－MS displayed a quasi－molecular ion at 885 $[M-H]^-$, together with ion peaks at m/z 753 $[M-132-H]^-$, 740 $[M-146]^-$, 445 $[M-132-146-162-H]^-$, which suggested that **2** contained rhamnose and xylose as terminal sugars, and glucose as inner sugar in the sugar sequence, and ophipogenin **4** to be aglycone. This was further confirmed by the $^{13}C$ NMR spectral data of **2** (Table 1) which, by comparison of those between **1** and **4**, obviously indicated that **2** possessed **4** as the aglycone moiety and had one more terminal xylose than that of **1**. By further comparison of the $^{13}C$ NMR data due to sugar moiety of **2** and **1**, it was observed that the chemical shift ascribed to C－3 of inner glucose was downfielded 10 ppm from δ 77.98 of **1** to δ 88.29 of **2**, and the chemical shifts due to C－2 and C－4 of inner glucose were also upfielded about 3 ppm, which revealed that one more xylose of **2** was glycosidated with C－3 hydroxyl group of inner glucose, thus the sequence of sugar was established to be －α－L－rhamnopyranosyl (1→2) [(－D－xylopyranosyl (1→3]－β－D－glucopyranosyl. By the way, the glycosidation shifts at C－2 (－1.4 ppm), C－3 (＋5.9 ppm), and C－4 (－3.5 ppm) were observed for the aglycone moiety by being compared with data of **4** (Nakanishi et al, 1987), which proposed that the sugar moiety be attached at C－3 hydroxyl group of

aglycone moiety. Consequently, the structure of **2** was deduced to be ophiogenin 3－O－α－L－rhamnopyranosyl (1→2) [β－D－xylopyranosyl (1→3] －β－D－glucopyranoside (**2**).

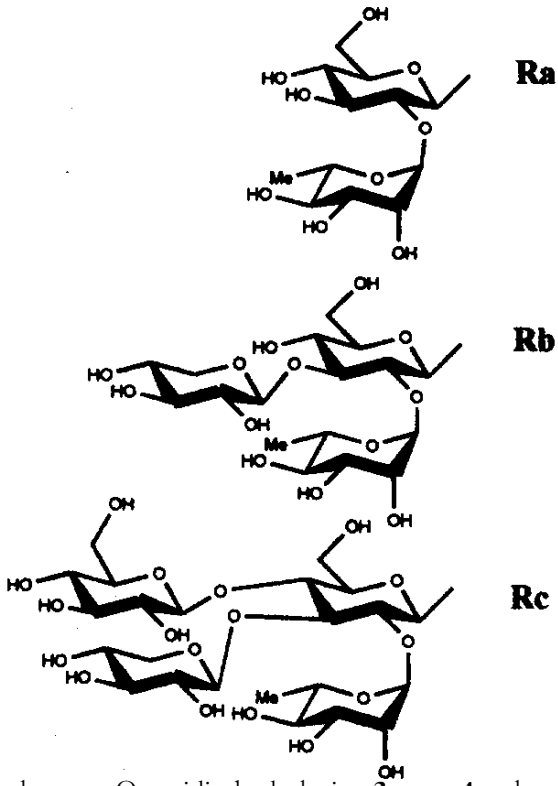
Table 1 $^{13}C$ NMR spectral data of glycosdie **1**～**3**

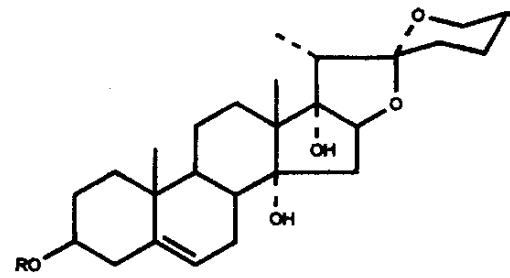
| | The aglycone moieties | | | | The sugar moieties | | |
|---|---|---|---|---|---|---|---|
| Carbon | **1** | **2** | **3** | Carbon | **1** | **2** | **3** |
| 1 | 37.83 | 37.80 | 37.80 | Glc－1′ | 100.44 | 100.03 | 100.03 |
| 2 | 30.30 (－1.2) | 30.17 (－1.4) | 30.17 (－1.4) | 2′ | 79.67 | 76.43 | 76.03 |
| 3 | 77.98 (＋6.4) | 77.53 (＋5.9) | 77.53 (＋5.9) | 3′ | 77.98 | **88.29** | **80.41** |
| 4 | 39.11 (－3.2) | 38.82 (－3.5) | 38.82 | 4′ | 72.90 | 69.62 | **80.40** |
| 5 | 140.44 | 140.34 | 140.40 | 5′ | 78.21 | 77.85 | 78.22 |
| 6 | 122.31 | 122.42 | 122.45 | 6′ | 62.82 | 62.49 | 62.17 |
| 7 | 26.28 | 26.22 | 26.25 | Rha－1″ | 102.06 | 102.31 | 102.16 |
| 8 | 36.34 | 36.35 | 36.37 | 2″ | 72.02 | 72.47 | 72.50 |
| 9 | 43.60 | 43.59 | 43.64 | 3″ | 72.58 | 72.90 | 72.85 |
| 10 | 37.48 | 37.46 | 37.49 | 4″ | 74.27 | 74.13 | 73.98 |
| 11 | 20.16 | 20.16 | 20.20 | 5″ | 69.48 | 69.62 | 69.98 |
| 12 | 26.61 | 26.62 | 26.65 | 6″ | 18.86 | 18.67 | 18.65 |
| 13 | 48.38 | 48.39 | 48.43 | Xyl－1‴ | | 105.43 | 102.68 |
| 14 | 87.82 | 87.83 | 87.87 | 2‴ | | 74.74 | 73.20 |
| 15 | 40.43 | 40.43 | 40.44 | 3‴ | | 78.37 | 78.75 |
| 16 | 90.57 | 90.58 | 90.60 | 4‴ | | 70.72 | 70.67 |
| 17 | 91.16 | 91.17 | 91.21 | 5‴ | | 67.28 | 66.91 |
| 18 | 20.67 | 20.16 | 20.72 | Glc－1⁗ | | | 102.68 |
| 19 | 19.45 | 19.44 | 19.48 | 2⁗ | | | 73.98 |
| 20 | 45.22 | 45.23 | 45.26 | 3⁗ | | | 76.05 |
| 21 | 9.73 | 9.74 | 9.77 | 4⁗ | | | 70.95 |
| 22 | 109.62 | 109.64 | 109.60 | 5⁗ | | | 76.93 |
| 23 | 32.11 | 32.22 | 32.25 | 6⁗ | | | 61.58 |
| 24 | 28.95 | 28.95 | 28.98 | | | | |
| 25 | 30.44 | 30.45 | 30.48 | | | | |
| 26 | 66.86 | 66.87 | 66.91 | | | | |
| 27 | 17.29 | 17.30 | 17.35 | | | | |

Glycosidation shifts given in the parentheses by being compared with reported values of ophiogernin (4); Assignments on the basis of $^1H-^1H$, $^{13}C-^1H$ Correlated 2D Spectroscopy (COSY) and Distortionless Enhancement by Polarization Transfer (DEPT). Glc：β－D－glucopyranosyl；Rha：α－L－rhamnopyranosyl；Xyl：β－D－xylopyranosyl.

Cixi－ophiopogon A (**3**) exhibited a molecular formula $C_{58}H_{70}O_{23}$ which was determined from the quasi－molecular ion peak at m/z 1047.4973 $[(C_{58}H_{70}O_{23})-H]^-$ in its negative ion HR－FAB－MS measurement. The IR of **3** demonstrated the typical absorptionof 25 (R)－spirosteroid at 980, 920, 900 and 870 $cm^{-1}$ (the intensity：900 > 920). The $^1H$ NMR spectrum of **2** possessed (See Experimental) similarity to that of glycoside **2** except for one more anomeric proton signal, which suggested that **3** was composed of **2** and one more hexose, and the coupling constants of the anomeric proton signals at δ 4.22 (1H, d, J = 8.0 Hz), δ 4.90 (1H, d, J = 8.1 Hz), 5.41 (1H, d, J = 8.0 Hz), and 6.16 (1H, br. s) implied the

presence of two β linkages and one α linkage in the sugar chain. Negative ion FAB－MS gave a quasi－molecular ion at m/z 1047 $(M-H)^-$ as well as ion peaks at m/z 915 $[M-132-H]^-$, 901 $[M-146-H]^-$, 885 $[M-162-H]^-$, 753 $[M-132-146-162-H]^-$, 445 [M－sugar chain]$^-$, which indicated that **3** was comprised by three terminal sugar, viz, glucose, rhamnose and xylose, and another sugar glucose as inner sugar in the sugar sequence, and **4** to




**1** R = Ra
**2** R = Rb
**3** R = Rc
**4** R = H

be aglycone. On acidic hydrolysis, **3** gave **4**, glucose, rhamnose and xylose by TLC and PC comparison with authentic samples. This was proved by the $^{13}C$ NMR spectral data of **3** (Table 1) which, by comparison of those between **2** and **4**, obviously indicated that **2** possessed **4** as the aglycone moiety and had one more terminal glucose than that of **2**. By further comparison of the $^{13}C$ NMR data due to sugar moiety of **3** and **2**, clearly proposed that **3** possessed **4** as aglycone moiety and had one terminal glucose than that of **2**. By carefully comparison of the $^{13}C$ NMR data of sugar moiety of **2** and **3**, it was noted that the chemical shift due to C－4 of inner glucose was downfielded 10 *ppm* from δ 69.62 of **2** to δ 80.41 of **3**, which revealed that the one more glucose of **3** was glycosidated with C－4 hydroxyl group of inner glucose. Enzymatic hydrolysis of **3** by β－glucosidase gave deglucosyl derivative which was identified as **2** by TLC comparison. Glucose in water layer was determined by TLC and PC comparison with authentic samples. Thus the sequence of sugar chain was characterized. Meanwhile, the glycosidation shifts at C－2 (－1.4 ppm), C－3 (＋5.9), and C－4 (－3.5) were also observed for the aglycone moiety by comparison of data of ophiogenin (**4**) (Nakanishi *et al*, 1987), which meant that the sugar moiety was attached at C－3 hydroxyl group of aglycone moiety. Hence,

the structure of **3** was thereof concluded to be ophiogenin 3－O－α－L－rhamnopyranosyl (1→2) [β－D－xylopyranosyl (1→3)] [β－D－glucopyranosyl (1→4)]－β－D－glucopyranoside (3).

## Experimental

Melting points were determined on Kofler hot stage apparatus and uncorrected. Optical rotation was measured with a J－20C digital polarimeter at room temperature. IR spectra were recorded in KBr on a Perkin－Elemer 577 spectrometer. FAB－MS was performed on a VG AUTOAPEC 3000 mass spectrometer. The NMR spectra were run on a Bruker AM－400 instrument at 400 MHz for $^1H$ and 100.6 MHz for $^{13}C$ in $C_5D_5N$, and chemical shits were given as δ (ppm) with TMS as an internal standard. Column chromatography was carried out on Qingdao silica gel, FUJI gel (ODS－$Q_3$) (Mitsubishi Chemical Co.), and Lichprep Lobar Rp－18 gel (Merck), TLC were performed on Merck precoated plates (Kiessel gel 60$F_{254}$ and Rp－18 $F_{254}$) with the following solvent system: A, $CHCl_3$－MeOH－$H_2O$ (8∶2∶0.2, v/v); B, MeOH－$H_2O$ (8∶2); and C, nBuOH－HAC－$H_2O$ (4∶1∶5, upper layer).

**Plant material.** The plant used in this research was collected in September 1995 in Cixi County, Zhejiang Province, China and was identified as *Ophiopogon japonicum* by Hangzhou Institute of Drug Control. The voucher specimen was deposited in this institute.

**Isolation of glycoside**. Root of *O. japonicum* (20 kg) was extracted with 90% EtOH under reflux for 3 times. The residue from the evaporation of solvent was dissolved in water and chromatographied on a $D_{101}$ resin with $H_2O$－EtOH (100 to 10%, v/v) to give 4 fractions. The 50% $H_2O$－EtOH eluent as main glycoside was concentrated in vacum to obtain 30 g of crude glycoside which was subjected to flash and medium pressure chromatography on silica gel with $CH_3Cl$－MeOH－$H_2O$ (95∶5∶0 to 80∶20∶0.2) to give fraction Ⅰ (mainly **1**), Ⅱ (mainly **2**) and Ⅲ (mainly **3**) followed by reverse－phase column chromatography on MCI CHP－20, Lichprep Lobar Rp－18 and FUJI gel (ODS－$Q_3$) with MeOH－$H_2O$ (7∶3 to 8∶2) to afford glycoside **1** (20 mg, 0.0002%), **2** (50 mg, 0.0005%), and **3** (200 mg, 0.002%).

**Ophiogenin 3－O－α－L－rhamnopyranosyl (1→2)－β－D－glucopyranoside (1).** An amorphous white powder; mp 248～252 ℃; $[\alpha]_D^{25}$ －93.33° (MeOH, c 0.3); FABMS (negative) m/z: 753 $[M-H]^-$, 607 $[M-146-H]^-$, 445 $[M-146-162-H]^-$; HR－FABMS (neg.) m/z 753.4035 [M ($C_{39}H_{62}O_{14}$)－H]$^-$, calcd 753.4062; IR $\nu_{max}$ $cm^{-1}$: 3480 (OH), 1630 (C＝C), 1060 (C－O－C), 980, 925, 902 (intensity 902＞925), 870; $^1H$ NMR ($C_5D_5N$) δ 0.67 (3H, d, J = 5.6 Hz, H－27), 1.10 (3H, s, H－18), 1.13 (3H, s, H－19), 1.27 (3H, d, J= 7.2 Hz, H－21), 1.79 (3H, d, J = 6.2 Hz, H－6′), 4.81 (1H, t, J = 7.6 Hz, H－16), 5.02 (1H, d, J = 7.8 Hz, H－1′), 5.39 (1H, br.s, H－6), 6.16 (1H, br.s, H－1″); $^{13}C$ NMR: see Table 1.

**Cixi－ophiopogon A (2).** An amorphous white powder, mp 250～255 ℃, $[\alpha]_D^{25}$ －39.09° (MeOH, c 0.55); FABMS (neg.) m/z: 885 $[M-H]^-$, 753 $[M-132-H]^-$, 740 [M－

146]$^-$, 445 [M－132－146－162－H]$^-$; HR－FABMS (neg.) m/z 885.4549 [M ($C_{44}H_{70}O_{18}$)－H]$^-$, calcd 885.4485; IR $\nu_{max}$ cm$^{-1}$: 3450 (OH), 1640 (C=C), 1060 (C－O－C), 980, 930, 910 (intensity 910>930), 870; $^1$H NMR ($C_5D_5N$) δ 0.65 (3H, d, J = 6.2 Hz, H－27), 1.09 (3H, s, H－18), 1.12 (3H, s, H－19), 1.26 (3H, d, J = 7.2 Hz, H－21), 1.74 (3H, d, J = 6.2 Hz, H－6″), 4.38 (1H, d, J = 8.1 Hz, H－1‴), 4.80 (1H, t, J = 7.6 Hz, H－16), 4.93 (1H, d, J = 7.8 Hz, H－1′), 5.30 (1H, br.s, H－6), 6.16 (1H, br.s, H－1″); $^{13}$C NMR: see Table 1.

**Cixi－ophiopogon B (3).** An amorphous white powder; mp 218～222 ℃; $[\alpha]_D^{25}$ －57.82° (MeOH, c 0.31); FABMS (neg.) m/z: 1047 [M－H]$^-$, 915 [M－132－H]$^-$, 901 [M－146－H]$^-$, 885 [M－162－H]$^-$, 753 [M－132－162－H]$^-$, 445 [M－132－146－162－162－H]$^-$; HR－FABMS (neg.) m/z 1047.4973 [M ($C_{50}H_{80}O_{23}$)－H]$^-$, calcd 1047.4988; IR $\nu_{max}$ cm$^{-1}$: 3450 (OH), 1640 (C=C), 1060 (C－O－C), 980, 920, 900 (intensity 900>920), 870; $^1$H NMR (C5D5N) δ 0.65 (3H, d, J = 6.2 Hz, H－27), 1.09 (3H, s, H－18), 1.10 (3H, s, H－19), 1.26 (3H, d, J = 7.2 Hz, H－21), 1.74 (3H, d, J = 6.0 Hz, H－6″), 4.22 (1H, d, J = 8.0 Hz, H－1‴), 4.80 (1H, t, J = 7.8 Hz, H－16), 4.90 (1H, d, J = 8.1 Hz, H－1′), 5.41 (1H, d, J = 8.0 Hz, H－1⁗), 5.40 (1H, br.s, H－6), 6.16 (1H, br.s, H－1″); $^{13}$C NMR: see Table 1.

**Enzymatic hydrolysis of 3 with β－glucosidase.** A suspension of 3 (10 mg) and β－glucosidase juice from Almond (0.2 mL) in 5 mL of 0.3 mol/L NaOAc buffer adjusted to pH 5.5 was allowed to stand at 37℃ for 3 days. Removal of solvent under reduced pressure gave a residue which was detected to contain glycoside **2**, and glucose by TLC ($CHCl_3$－MeOH－$H_2O$, 8∶2∶0.2 and 4∶3∶1 on silica plate, and MeOH∶$H_2O$, 8∶2 on Rp－18 plate) and PC (nBuOH－HAc－$H_2O$, 4∶1∶5, up－layer) analyses.

**Acidic hydrolysis of glycoside 1, 2 and 3.** A soln of **1** (5 mg) in 1 mL HCl－MeOH (2 mol/L) was refluxed at 100 ℃ for 2 hrs and then neutralized with sat. $Ba(OH)_2$ aq. Removal of MeOH followed by participation between $H_2O$ and $CHCl_3$. The ophiogenin (**4**) was detected from the $CHCl_3$ layer by TLC comparison. Glucose and rhamnose in water were revealed by TLC and PC analyses. The other glycoside **2** and **3** were acid－hydrolyzed with the same method described above to provide aglycone **4** from organic phase, and glucose, rhamnose and xylose from water layer.

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## References

江苏新医学院编, 1977. 中药大词典[M]. 上海: 上海科学技术出版社, 1024

朱永新, 严克东, 涂国士, 1987. 麦冬中高异黄酮的分离与鉴定[J]. 药学学报, 22(90): 679～684

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Adinolfi M, Parrilli M, Zhu YX, 1990. Terpenoid glycosides from *Ophiopogon japonicus* root[J]. *Phytochemistry*, **29**(5): 1696～1699

Branke J T, Hashilinger E, 1995. Spirostanol glycoside from the tuber of *Ophiopogon japonicus*[J]. *Liebigs Ann*, 587～589

Nakanishi H, Kameda N, 1987. Studies on the component of *Ophiopogon* tuber (China) II[J]. *Yakugaku Zasshi*, **107**(10): 780～784

Tada A, Kasai R, Saitoh T *et al*, 1980. Studies on the constituents of *Ophiopogonis* tuber. V. Isolation of novel class of homoisoflavonoids and determination of their structures (1)[J]. *Chem Pharm Bull*, **28**(6): 1477～1484

Tada A, Kasai R, Saitoh T *et al*, 1980. Studies on the constituents of *Ophiopogonis* tuber. VI. Structures of homoisoflavonoids (2)[J]. *Chem Pharm Bull*, **28**(8): 2039～2044

Tada A, Kobayashi M, Shoji J, 1973. Studies on the constituents of *Ophiopogonis* tuber. III. On the structures of ophiopogon D[J]. *Chem Pharm Bull*, **21**(2): 308～311

Tada A, Shoji J, 1972. Studies on the constituents of *Ophiopogonis* tuber. II. on the structures of ophiopogon B[J]. *Chem Pharm Bull*, **20**(8): 1729～1734

Watanabe Y, Sanda S, Tada A *et al*, 1977. Studies on the constituents of *Ophiopogonis* tuber. IV. On the structures of ophiopogon A, B′, C, C′, and D′[J]. *Chem Pharm Bull*, **25**(11): 3049～3055